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UNIVERSITY OF TORONTO STUDIES

PHYSIOLOGICAL SERIES

No. 7: THE DISTRIBUTION OF FAT, CHLORIDES, PHOSPHATES, POTASSIUM AND IRON IN STRIATED MUSCLE,

BY MAUD L. MENTEN

(REPRINTED FROM TRANSACTIONS OF THE CANADIAN INSTITUTE, VOL. VIII)

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THE DISTRIBUTION OF FAT, CHLORIDES, PHOSPHATES, POTASSIUM AND IRON IN STRIATED MUSCLE.

By MAUD L. MENTEN, B.A., M.B.

(Read 30th January 1909.)

UNTIL comparatively recently definite knowledge concerning the distribution of inorganic and organic compounds in muscle fibre had been somewhat meagre. This is due, for the most part, to the fact that many of the microchemical methods have been only lately introduced into histological study; then, too, the small amounts in which these substances, and the mineral salts especially, occur, and the minute structure of striated muscle also render their demonstration difficult.

In the following pages are detailed a series of observations made during the last two years on the microchemistry of muscle fibre, chiefly of forms amongst the Insecta and Crustacea. These observations are, unfortunately, not at all as comprehensive as the author desired, but they may prove of service to other investigators and they are now, in consequence, put on record.

I—PREVIOUS OBSERVATIONS.

In 1899, Walbaum[1] studying the muscle of rachitic children for evidence of fatty degeneration, came to the conclusion that fat could occur in the muscle tissue independent of any pathological condition. In the latter part of his work not only did he find fat occurring in muscle which was apparently normal, but he was also able to demonstrate a more or less definite relation to the cross striation. This latter was especially evident in the eye muscles, in which the size of the fat droplet was proportional to the width of the striation. Using Sudan III and Hæmatoxylin staining Keinath[2] found large droplets of fat in proximity to the nuclei of the muscle, and fairly constantly grouped about either of their poles. In the striated muscle fibres of animals in almost every case fat arranged as droplets in longitudinal rows between the fibrils was distinctly discernible and in the muscles of the goat and dog the fibrils themselves contained fat in the form of very fine granules but in varying numbers and sizes and with no definite arrangement that could be recognized. In human muscle which was normal, and in the pathological where the structure of the

fibril was not destroyed, he obtained similar results, but in no case was he able to discover that the fat bore any relation whatever to the cross striation.

Of the mineral constituents of the muscle, the only element whose distribution has been studied microchemically is potassium. Macallum[4] has shewn that the salts of this metal possess a definite arrangement in the fibrils of uncontracted striated muscle, being limited to the dim band in the wing muscles of insects and the claw muscles of the crayfish but the potassium reaction was most marked in those zones of the dim band which are adjacent to the light bands. In the contracted fibril on the other hand he found that the most marked reaction for potassium was given by the central zone of the dim band. When, however, the penetration of the reagent (the hexa-nitrite of cobalt and sodium) is tardy there occurs a redistribution of the potassium salts in the fibres and in consequence the potassium demonstrated was sometimes in the light bands alone and sometimes along the line of separation between two adjacent light and dim bands.

II—THE LOCALIZATION OF THE FAT.

To show the microchemical distribution of fat the reagent used was Scarlet Red, a stain belonging to the Tetrazo Compounds. This substance was introduced into microchemistry by Michaelis who studied its composition and properties, and by whom it was classed in the group of indifferent staining materials, which also includes Sudan III. It is insoluble in water, alkalies and acids, with the exception of concentrated sulphuric acid with which it gives a blue colour, soluble in alcohol and is readily dissolved in the fats. Its action is very much more extensive than osmic acid, which reacts only with unsaturated fatty compounds, such as oleic acid and olein, yet it stains nothing but the fats, whereas the osmic acid may be secondarily reduced by substances other than the oleic compounds, and the resulting black precipitate may represent more than the distribution of the fat.[5] Michaelis explains its action by the fact that it belongs to the indifferent substances, i.e., those possessing no salt-forming group and whose acid or basic properties are so weak as to be practically negligible and, therefore, the staining process is a physical rather than a chemical one, but that in order to be soluble in the fat the molecule of the staining compound must have a characteristic chemical composition. This latter statement seems to be borne out by the observation that palmitic and stearic acid crystals stain with some difficulty by Scarlet Red and

Sudan III at ordinary temperatures, but when either of these fats is heated to the melting point in contact with the fat stain the whole becomes a deep red. However on recrystallization on cooling the majority of the crystals fail to retain their coloration. Mann[6] does not accept the physical explanation for the staining of the fatty materials, and suggests that the process is rather one of oxidation.

The solution used was one prepared by dissolving the Scarlet Red powder in 70 per cent. alcohol and allowing it to stand several days. Fischer[7] claims that a solution made with 70 per cent. alcohol at the boiling point or leaving the solution in a parrafin oven for 24 hours, produced better results in a much shorter period of time. However, in my experiments the additional heating apparently had little effect in accelerating the staining and both solutions required, comparatively speaking, the same time to give like results.

Before using the solution all sediment should be removed by filtration. Any excess of the stain may be washed off with distilled water, as washing in alcohol, even if of a weak dilution, dissolves out more or less of the fat, and it is, therefore, not to be recommended. The muscle tissue which was found most advantageous for these microchemical investigations was that of insects, owing to the size of the fibrils and their greater development. In many cases fresh tissue was used while in other experiments the muscle was previously fixed in 4 per cent. formaline or a 5 per cent. solution of chloral hydrate. Previously to being placed in the Scarlet Red, the fibres must be thoroughly separated so as to facilitate the penetration of the reagent to every part. While a reaction obtains in ten to fifteen minutes it appears only in the larger fat droplets or those most readily accessible to the staining fluid and the best results, both in detail and distinctness, were obtained by leaving the tissue in the solution for five days. When allowed to remain longer than this in the staining fluid no further trace of fat was revealed, and apparently the reaction was not in any way increased.

When muscle fibres treated according to the methods outlined above are examined microscopically the fibrils are seen to be transversely marked by bright red bands, occurring at regular intervals throughout the entire length of the fibre, and under a high magnification these striations are found to be beaded or made up of fine granules, which here and there have coalesced to form small globules. These striæ lie in the dim band, one being along Hensen's line, across

the centre of the dim band and another on either side of this at its upper and lower borders. In some case Hensen's line appears as an exceedingly fine faintly colored thread (Fig. 3) and as the particles which compose it are often so fine as to be only discernible in the ultramicroscope, some conception may be had of their minute size. In many cases, however, the line appears equal in width to those on either side of it (Figs. 4 and 5).

That this substance is undoubtedly fat is shewn by the fact that the fat solvents readily remove it from the fibres and if these are employed previously to the staining of the tissue with the Scarlet Red either no indication of the reaction appears, or one of very much lessened intensity depending on the time the solvent has been in contact with, and how readily it has penetrated the muscle fibres. For the purpose of demonstrating this, muscle previously fixed in formol was treated first with 70 per cent. alcohol for four hours then with absolute alcohol for twelve to twenty-four hours and finally with ether for from twenty-four hours to one week. The preparations were afterwards repeatedly washed with alcohol until every trace of ether was removed from them. Subsequent treatment with Scarlet Red fails to reveal any trace of a colored striation if the tissue has been sufficiently teased to allow a complete penetration of the ether.

Where the tissue has not been thoroughly separated, although the muscle has remained in the ether for several days, the fat is not completely dissolved out and the fibrils give on treatment with Scarlet Red a reaction characteristic in its distribution but of a much fainter colour. The final extraction of the fat occurring normally in the muscular tissue, is as Bogdanow[8] has shewn very difficult to obtain. He found that even after repeated extractions in a Sohxlet apparatus with ether he could obtain a reaction with osmic acid in the muscle fibre.

When the myoplasm surrounding the fibril has not been removed by the teasing, it stains a deep homogeneous reddish orange (Fig. 2) which apparently indicates a diffuse distribution of the fat in that structure.

The distribution of the fat is not constant in any one part of the fibre alone. Most frequently it appears in the dim band, but it may occur only in the light band, and, occasionally, simultaneously in both. This variation seems to be intimately connected with the activity of the muscle, i.e., whether the muscle is in the resting or contracting stage.

In the majority of the preparations studied the reaction is confined to the dim band, which is marked by regularly occurring longitudinal lines of a beaded character (Figs. 7 and 8). The droplets are usually of uniform size and depth of colour, and in some instances they form a continuous and unbroken line throughout the length of the dim band (Fig. 8) while in others there is a tendency for the fat to be aggregated in the outer zones of the dim band, leaving a narrow space immediately in the centre of the band, which is absolutely free from any trace of the scarlet stain (Fig. 7). This massing of the fat towards the poles of the dim band may advance yet farther until the fat appears at the extreme edges of the dim band with occasional faintly colored droplets in the central part (Fig. 8).

Frequently, however, the fat is wholly limited to the light band (Fig. 10). An approximation to this condition is shewn in Fig. 9 where the greater part of the fatty material lies in the outer thirds of the light band, but an occasional trace is also visible in the dim band. The striated character of the reaction is to be observed in the fat occurring in the light as well as in the dim band. The granules are visible in both cases and they always occur in longitudinal lines. Among those fibrils which had been subjected to treatment with Scarlet Red for five or more days occasionally was to be seen a result as represented in Fig. 11, where a granular longitudinal striation appeared in the light band, and an additional thin line of fainter colour, occurred in the dim band marking the position of Hensen's line. The longitudinal granular character which is so distinctly marked in the dim band is maintained in the reaction along Hensen's line. In the centre of the light band, extending horizontally across it, along Dobie's line, is a narrow space devoid of any coloration.

A clue to the explanation of these diverse pictures of the distribution of the fat is obtained from those fibres which have been fixed while a wave of contraction was passing over them (Fig. 12). In the lower contracted part of the fibril the fat lies wholly in the dim band, the centre of which is devoid of any reaction in contrast with the deeply stained red granules, regularly arranged in longitudinal lines in the zone on either side of this. In the upper part, where the fibre is uncontracted the dim bands shew only a diffuse reaction pale in colour with the fat in granules arranged along the borders adjacent to the light bands. The assumption, therefore, is that Fig. 7 represents the distribution of the fats in complete relaxation and Fig. 11 that of complete contraction, and the various intermediate stages are indicated in Figs. 8, 9, and 10.

Results quite similar to those obtained with Scarlet Red were obtained also with another fat staining reagent. J. Lorrain Smith[9] after a somewhat extensive study of the action of the different basic dyes, including Methyl Violet, on fatty acids and numerous fatty compounds, concluded that the reaction between the fat and the dye which he obtained was due to the formation of a colored soap. In that case Methyl Violet should demonstrate in muscle tissue the same distributiou of fat as was observed when Scarlet Red was used and to test this a number of experimental observations were made. Part of the muscle tissue, which had been previously fixed in 4 per cent. formalin or 5 per cent. chloral hydrate solution, and from which fibres treated with Scarlet Red gave characteristic results as described in the foregoing pages, was stained in Methyl Violet. The result was in every way comparable to that obtained with the Scarlet Red except that in these latter experiments the fats were colored violet instead of red. When tissue fixed in absolute alcohol was used, if the fibres were thoroughly separated a very faintly colored reaction resulted.

It was further, found that if the fibres were left in ether for several days, (using a technique in every way similar to that already mentioned in the Scarlet Red experiments with ether extraction) and then stained with the Methyl Violet, the fibrils where they had been sufficiently separated by teasing, shewed a pale mauve colour, barely perceptible in the striations. The Methyl Violet undoubtedly stained a substance with a distribution analogous to that which gave a reaction with the Scarlet Red and which further was soluble both in alcohol and ether.

Whether other constituents of the muscle fibre which are soluble in ether stain with Methyl Violet cannot at present be determined. One compound, creatin, present in the dim bands is soluble in water and alcohol but is not soluble in ether and therefore it does not play a part in the fixation of the dye.[10]

That Methyl Violet does stain fats very deeply was shown in the case of lecithin. When glass slides were smeared with a thin film of this fat, treatment for a few minutes with a solution of the dye gave an intensely deep violet coloration. When similar films were treated with solutions of Scarlet Red the resulting red stain was equally intense. With other fats, e.g., olive oil, the reactions were similar and as pronounced.

III—THE LOCALIZATION AND THE DISTRIBUTION OF THE CHLORIDES.

In the various analyses of the inorganic constituents of muscle from different animals there is shewn a marked variability in the amount of the chlorides. This chloride content is especially high in the invertebrates and Henke[11] found on estimation of that mineral in Octopus as much as 2.7977 grams in 100 parts of the dried muscle. It is remarkable, too, that in the table of percentages, which Katz[12] gives as the result of his analyses of various muscle tissues, the quantitative amount of chlorides in invertebrate muscle is so much greater than that in the muscle of the vertebrates.

The salt water shell-fish examined by him gave 1.2477 per cent. of chlorine (dried muscle) in comparison with 0.0935 per cent. and 0.3415 per cent. which represent respectively the minimal and maximal amounts obtained from the vertebrate muscle. This excess cannot be explained wholly by the fact that chlorine forms one of the preponderating elements in the medium in which they live, for if so, a similarly high percentage would be found but was not observed in the muscle of the marine fishes, which he analyzed.

In all estimations of the chlorine content of muscle it is a question whether any or all of the chlorine found is localized in the fibre, that is, within its sarcolemma and if within the fibre in what portions of each fibril. The observations now detailed bear on the latter part of this problem.

The method of studying the distribution of the chlorine of chlorides was the same as that already used by Macallum and Menten,[13] to shew the distribution of the chlorides in the nerve fibre, and a detailed description of the method appeared in the account of that work. The reagent used was a decinormal solution of silver nitrate, to which was added enough of nitric acid to give the reagent 1.5 per cent. of the acid The reagent must be prepared with water free from any trace of chlorides, and the tissue should be teased with glass or quill points to preclude the possibility of any contamination with the same impurity.

To obtain good results the fibrils must be completely separated as the reagent penetrates the tissue comparatively slowly when in a mass. The best preparations were obtained after an immersion in the nitrate

solution for three days, but usually a few hours sufficed to give forms showing typical distributions.

When sufficiently acted upon by the reagent the muscle fibres were mounted directly on a slide in 50 per cent. glycerine, and placed in direct sunlight for several hours. Teased out thorax muscle fibre of insects which has been for four or five days in contact with a solution of the silver nitrate containing nitric acid, and then exposed to the sunlight, shews the fibrils marked by a transverse striation similar to that obtained in the preparations demonstrating the distribution of the fat. The striae are granular, of a deep brown colour and they occur at regular intervals throughout the entire length of the fibre (Fig. 16). In addition to the deeply colored brown precipitate a diffuse reaction is frequently noticed in the light bands, perhaps in part due to a more rapid penetration of the nitric acid, and a consequent slight redistribution of the chlorides.

That the chlorides are not limited to the peripheral layers of the fibre, but extend in horizontal planes across it, may occasionally be observed in favourable preparations (Fig. 18). The granular character of the precipitate is distinctly recognizable, individual granules being readily discernible. On careful examination the division into dim and light bands is easily distinguished, and the chlorides are seen to be localized in the dim bands. At its upper and lower edges near the junction with the light bands. The reaction in this part of the fibril is very intense, and on prolonged treatment with silver nitrate a third striation frequently appears also in the dim band, but this is of a much fainter colour. This additional striation lies exactly in the centre of the band, marking the position of Hensen's line (Fig. 17).

Although the muscle tissue, unless in a finely divided condition, is very slowly penetrated by the reagent the reaction may occasionally be observed in a considerable number of contiguous fibres, which have not been separated and in these cases the striæ appear as discs extending horizontally across several fibres (Fig. 19).

The distribution of the chlorides in the dim band, however, is more readily demonstrated in the wing muscles. The whole fibre is marked by many longitudinal striae (Fig. 20) lying along definite straight lines and parallel to one another. At the first glance, it appears as if these regularly distributed, deep brown lines were continuous throughout the entire length of the fibre, but a careful examination reveals the fact

that there is an interruption in the light band which rarely contains any of the darkened sub-chloride of silver. Each stria is composed of fine granules, which are the colored particles.

The chlorides, however, are not always confined to the dim band, and although amongst the preparations studied, this seemed to be the distribution, which most frequently obtained, the reverse picture is often met with, the chlorides being aggregated at the junction of the light and dim bands, while the dim band itself appears comparatively free from any reaction. In Fig. 22, for example, the silver salts are shown massed along the upper and lower border lines of the light band, and only a few scattered granules of a faint colour appear to lie in the dim band. Fig. 21 represents the chlorides occurring simultaneously in both the light and dim elements of the muscle fibre, and while the greater part of this inorganic material appears in regularly distributed vertical lines in the dim band, frequently these lines are continued into the light band.

Since the reaction may be well marked on the borders of the light band and the dim band be devoid of any coloration, and vice versa, the result can hardly be due to a redistribution caused by the advance of the penetrant acid being more rapid than that of the silver nitrate for in these cases a diffuse reaction also occurs. Hence it is inferred that there is an alteration or rearrangement in the disposition of these salts, which has some definite relation to the activity of the muscle.

It can scarcely be doubted but that these various striations represent the arrangement of the chlorides as they obtain in the living muscle. It is true that striæ may result (as in the Boehm-Liesegang phenomena) from a uniform distribution of inorganic salts in solutions of definite consistency as has been experimentally shewn by treating mixtures of albumen and of gelatine in glass tubes or plates with certain silver salts and then exposing the preparations to the sunlight. In these cases, however, the striæ and the interstriate zones are of varying widths, being wider and less sharply defined as the reaction progresses in the direction of the line of diffusion ; also the depth of the reaction in each gradually decreases from the central point peripherally. In the muscle, on the other hand, the tone of reaction throughout the individual fibres is constant and the striations are sharply defined. When variations in the width of the striæ do occur they may be explained as due to the differences in the width of the dim bands.

The distribution of the chlorides in striated muscle, therefore, as evidenced in the preparations obtained may occur either in the dim band chiefly, or alone, or in the light and dim band, the distribution depending, apparently, upon the phase of activity existing in the fibril at the moment of fixation.

IV—THE LOCALIZATION AND THE DISTRIBUTION OF THE POTASSIUM

An exceedingly sensitive reagent to shew minute traces of potassium was introduced into microchemistry by Macallum[14] in his work on the distribution of that element in plant and animal tissue. This compound, the hexanitrite of cobalt and sodium, $Co\ Na_3\ (No_2)_6$, with even a minute quantity of potassium either free or in combination gives an orange-yellow precipitate, which is rendered more evident by adding ammonium sulphide, after the tissue has been washed from every trace of the uncombined cobalt salt. The resulting black sulphide indicates the distribution of the potassium. The method of preparing the reagent employed was the same as that used by the above mentioned author, and full details are given in the account of his work. It is prepared by dissolving twenty grammes of cobalt nitrite and thirty-five grammes of sodium nitrite in seventy-five c.c. of water containing ten c.c. of glaciol acetic acid. The solution was then diluted to 100 c.c. In the early part of the work the reagent used was of this composition, but owing to its somewhat slow penetrability into the muscle fibrils, better results were subsequently obtained with a solution diluted with a third or one-half of a fifty per cent. solution of sodium nitrite. Care was taken that every trace of the uncombined cobalt reagent was removed from the tissue before it was treated with the ammonium sulphide, as otherwise a reaction would take place between the uncombined cobalt salt and the sulphide, thus giving a black precipitate. The preparations were mounted in fifty per cent. glycerine.

From the various analyses of striated muscle, potassium forms one of the predominating elements amongst the inorganic constituents and because of this preponderance the tissue readily lends itself to microchemical investigation of this metal, which gives most distinct and characteristic results. Macallum has already shewn that these salts are definitely distributed in muscle. The same author has also pointed out that the cobalt reagent gives with creatin a constituent of vertebrate muscle, a precipitate similar to that obtained with the potassium salts.

To obviate the difficulty arising from this fact all the experiments here detailed were carried out upon insect muscle, which is free from that compound or possesses it only in infinitesimal quantities. The reaction in the tissue is in all cases immediate, the maximum result being obtained in 3 to 5 minutes. Leaving the muscle fibres in contact with the reagent for a longer period of time in no case intensified or enhanced the results.

The tissue must be fresh and thoroughly teased-out, and for this purpose glass points or quills were used. The teased-out material, without further treatment, was then placed in the cobalt solution for five to fifteen minutes, washed thoroughly in ice-cold water, and mounted on a slide in equal parts of glycerine and concentrated ammonium sulphide. The wing muscles of insects shew the distribution of this salt in a remarkable manner and throughout a marked resemblance is observed to the results obtained with the reagents for the chlorides and fats. The potassium as in the case of the two above mentioned muscle constituents may be restricted to either the dim or to the light band, or it may occur simultaneously in both. When the dim band alone is affected a longitudinal striation is more or less clearly marked (Figs. 24-25) and this may possess a granular appearance extending continuously through the dim band (Fig. 25) or it may be limited to the two zones forming the upper and lower third of this band while the central third is comparatively free (Fig. 26). The striations when restricted to these latter situations, i.e., the upper and lower thirds of the dim band, do not possess a granular character, or if so, it is difficult to distinguish.

In Fig. 24 a marked condensation of the potassium is evidenced along the extreme border line of the dim band, and also midway between these, along Hensen's line.

When the potassium is localized in the light band apparently, it may occur in the longitudinal striation, an example of which is represented in Fig. 28, or it may be irregularly disposed (Fig. 30) without any trace of striation. An approximation to the restriction of this inorganic material to the border of the light band is occasionally met with (Fig. 27) where, many of the striations are confined entirely to this part of the fibre. Such preparations afford conclusive evidence of the change in position of the inorganic constituents. That this redistribution is intimately connected with the activity of the muscle seems apparent from such fibres, as those represented in Fig. 31, where a wave of contraction is advancing upward over the muscle. In the lower part

of the figure the potassium is isolated in the dim band, in the upper contracted part it occurs in the vicinity of the light band and between the two extremes the intermediate grades are obtained.

As already mentioned a comparison of the distribution of the chlorine of chlorides with that of potassium in muscle reveals a remarkable similarity in the disposition of the two, hence the inference that in striated muscle they possess an analogous distribution.

Also to be noted is the ease with which the fibril may be broken across at the light band, when it is free from any reaction. This structure may become so reduced in size as to be scarcely discernible except where the fibrils have become divided transversely (Fig. 25) through pressure on the cover glass.

V—THE DISTRIBUTION OF THE PHOSPHATES.

If fresh tissue, after having been thoroughly teased, be left in a decinormal solution of silver nitrate, the phosphates, chlorides and some other constituents of muscle which unite to form insoluble compounds with silver are precipitated, and in the sunlight these compounds are reduced, and assume a deep brown colour. The reagent should be prepared from distilled water free from any chemical impurity and every precaution should be observed to prevent the tissue becoming chemically contaminated. In these experiments as in those demonstrating the chlorides and potassium only glass or quill points should be used for teasing purposes. A characteristic reaction is obtained when the fibres have been in the reagent about three days, after which the tissue is then mounted in 50 per cent. glycerine and exposed to the action of direct sunlight.

The compounds in muscle fibre other than the chlorides and phosphates which unite with silver to give "reduced" silver products in sunlight, are probably present only in infinitesimal quantities and thus when the reagent is allowed to act on muscle fibre in the manner described the result is due to "reduced" silver phosphate and chloride. If the preparations before exposure to sunlight are treated with dilute nitric acid the "reduced" phosphate does not appear subsequently. Consequently, by comparing preparations made to show the chlorides alone with those treated with nitrate of silver but not with dilute nitric acid, one is enabled to determine, not strictly and definitely, but on the whole approximately, the distribution of the phosphates. By compar-

ing Figs. 32-38, which illustrate the phosphate reaction, with Figs. 16-22 which represent the distribution of the chlorides, one may determine what the localization of the phosphates is.

Next to potassium the phosphates are the most abundant inorganic constituents of striated muscle. Consequently the microchemical determination of their distribution is important. Invariably results analogous to those obtained in the chloride and potassium investigations, show the distribution of the phosphates to be the same as in the case of the chlorides and potassium salts. A most clearly marked precipitate possessing a granular character occurs along the edges of the dim bands, and a more faintly colored reaction at the central part indicates Hensen's line (Fig. 34). This represents a typical distribution and when, as in Fig. 33, the phosphates seem to occupy the whole light band, it is to be explained by the fact that this structure has become so narrowed that its appearance is obliterated by the juxtaposition of the lines of the precipitate which really occur in the edges of the dim band. The disposition of the phosphates in the wing muscle, too, bears a striking resemblance to that already demonstrated in the case of the chlorides and the potassium. Comparing Fig. 28 with Fig. 37, which respectively represent the distribution of the potassium and the phosphates on the borders of the light band, one sees the same localization obtaining in both, and further in Fig. 22, which represents the distribution of the chlorides there is an approximation to the same condition.

A comparison of Figs. 21 and 35 likewise reveals an analogous disposition of the chlorides and phosphates in the dim band, and the potassium shews a strikingly similar arrangement in Fig. 25.

Occasionally in the wing muscle fibres there is a tendency which is also met with in the potassium preparations, towards an irregular distribution of these salts in the light band. This departure from a regular arrangement of these inorganic constituents has been observed only in this part of the fibril, never in the dim band where the precipitate always possesses a distinctly longitudinal striation, and usually a definite granular character.

From the foregoing observations it is apparent that the chlorides, the phosphates and the potassium have an analogous distribution and it is justifiable, therefore, to conclude that the definitely localized arrangement displayed by all of them is associated in some way with activity of the muscle.

VI—THE IRON IN MUSCLE FIBRE.

For the demonstration of iron in the muscle fibres the reagent employed was hæmatoxylin, which has been shewn by Macallum to be a very sensitive one to determine the presence of iron in an inorganic combination. When inorganic compounds are treated with a 0.5 per cent. aqueous solution of hæmatoxylin the iron is represented by a blue-black reaction, but if the iron is present in the organic form no change in colour occurs. This latter may be converted into the inorganic form by the action of acid alcohol and when this is treated with the hæmatoxylin it gives the blue-black reaction above referred to. The acid alcohol used for this purpose was one containing four volumes of concentrated sulphuric acid in 100 parts of absolute alcohol in which the tissue was left for at least fifteeen hours at a temperature of 35° C. This tissue was previously fixed in a four per cent. solution of formaline and, after all the formaline was removed by washing thoroughly in alcohol, was transferred to the sulphuric acid alcohol in which the preparation remained for fifteen to twenty-four hours at 35° C. All traces of the acid were then removed by washing with absolute alcohol and the fibrils finally stained in the hæmatoxylin solution for thirty minutes. They were then mounted in 50 per cent. glycerine.

Using the aqueous hæmatoxylin alone on the fresh muscle tissue or on that previously fixed in alcohol or formaline there appeared in the fibrils only a slight yellowish brown coloration with a somewhat deeper colour in the narrow light band. The nuclei were stained a like diffuse yellow colour but with extremely fine darker granules scattered throughout (Fig. 13).

Apparently the muscle fibril and the nucleus contain, if any, only an infinitesimal amount of inorganic iron. When the muscle has been treated with the acid alcohol and then subjected to staining with aqueous hæmatoxylin solution a general, diffuse, faint purplish tone obtains in the dim bands, with more deeply stained, extremely fine granules irregularly distributed through them and what occupies the position of the narrow light band presents a very deep purplish colour (Fig. 14). In the nuclei the reaction is most intense especially along the chromatin threads (Fig. 15). When the myoplasm in which the fibril is embedded is not removed by the teasing, it takes a deep purple colour, equal in intensity to that obtaining in the light band, and the

reaction manifested in the latter indicates a diffuse distribution of the organic compounds of iron in that structure.

Since the depth of colour in vicinity of the light band approaches in intensity that in the chromatin threads, it is inferred that the amount of organic iron in this locality is a little less than in the chromatin. Since, however, the material used for this purpose was rather limited in quantity it is perhaps best not to insist too much on an interpretation of the phenomena observed.

In conclusion I wish to express my sincere thanks to Professor A. B. Macallum, for suggesting the subject of this research, and for his supervision throughout the course of the investigations.

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EXPLANATION OF FIGURES ILLUSTRATING THE MICROCHEMISTRY OF STRIATED MUSCLE.

Fig. 1.—Thorax muscle, Bee. Fixed in 4 per cent. formalin and stained in hæmatoxylin and eosin. The more deeply stained sheaths enclosing portions of the fibrils and fragments of the adherent myoplasm × 1000.

Figs. 2, 6.—Thorax muscle, Bee. Fixed in 4 per cent. formalin and stained in Scarlet Red, 10 to 14 minutes. In Fig. 2, the part of the fibre stained orange represents the myoplasm × 1000.

Fig. 7.—Wing muscle, *Tenebrio obscurus*. Fixed in 2 per cent. formalin stained with Scarlet Red, 10 minutes × 1000.

Fig. 8.—Wing muscle, *Tenebrio obscurus*. Fixed in 2 per cent. formalin and treated with Scarlet Red, 75 hours, shewing the fat confined to the dim band × 1000.

Fig. 9—10.—Wing muscle, *Tenebrio molitor*. Fixed in [illegible] per cent. formalin and stained with Scarlet Red, 75 hours. In both the fat occurs [illegible] in the light bands × 1000.

Fig. 11.—Wing muscle, *Tenebrio molitor*. Fixed in 2 per cent. formalin stained with Scarlet Red, 125 hours. A deep reaction obtains in the light band, but the central zone, which marks the position of Dobie's Line is free from fat. In the dim band a faint reaction occurs along Hensen's Line × 1000.

Fig. 12.—Wing muscle, *Tenebrio obscurus*. Fixed in 2 per cent. formalin and stained in Scarlet Red, 125 hours, shewing a fibre partly relaxed and partly contracted × 1000.

Fig. 13.—Thorax muscle, Bee. Fixed in 4 per cent. formalin, stained with 2 per cent. aqueous hæmatoxylin 36 hours. × 1000.

Fig. 14.—Thorax muscle, Bee. Fixed in 4 per cent. formalin and treated with absolute alcohol, and then sulphuric acid alcohol, 24 hours, stained with 2 per cent. aqueous hæmatoxylin × 1000.

Fig. 15.—Preparations of same shewing the reaction for organic iron which obtains in the nuclei × 1000.

Fig. 16.—Muscle, *Lachnosterna fusca*. $\frac{N}{10}$ $AgNO_3$ + 1.5 per cent. HNO_3, 48 hours, × 1000.

Fig. 17.—Muscle, Wasp. $\frac{N}{10}$ $AgNO$ + 1.5 per cent. HNO_3, 5 days. The faintly marked reaction represents Hensen's Line × 1335.

Fig. 18.—Muscle, *Lachnosterna fusca*. $\frac{N}{10}$ $AgNO_3$ + 1.5 per cent. HNO_3, 48 hours, showing the distribution of the chlorides extending in horizontal plane across the fibre × 1000.

Fig. 19.—Muscle, *Lachnosterna fusca.* $\frac{N}{10}$ $AgNO_3$ + 1.5 per cent. HNO_3, 48 hours × 1000.

Fig. 20.—Wing Muscle of *Calosoma Scrutator.* $\frac{N}{10}$ $AgNO_3$ + 1.5 per cent. HNO_3, 5 days × 1000.

Fig. 21.—Wing Muscle, Wasp. $\frac{N}{10}$ $AgNO_3$ + 1.5 per cent. HNO_3, 5 days × 1000.

Fig. 22.—Wing Muscle, Wasp. $\frac{N}{10}$ $AgNO_3$ + 1.5 per cent. HNO_3, 18 hours, shewing the distribution of the chlorides at the extreme edge of the dim band, and at the border of the light band × 1000.

Figs. 23-31.—Preparations treated with cobalt solution, $Co\,Na_3\,(NO_2)_6$, and ammonium sulphide.

Fig. 23.—Wing Muscle, *Tenebrio molitor.* Cobalt solution 67 parts + 50 per cent. sodium nitrate solution, 33 parts, 5 minutes × 1000.

Fig. 24.—Wing Muscle, *Calosoma Scrutator.* Cobalt solution 50 parts + 50 per cent. sodium nitrite solution 50 parts, 2 hours, shewing an aggregation of potassium at the border of the dim band, and along Hensen's line × 1000.

Fig. 25.—Wing Muscle, *Tenebrio Obscurus.* Cobalt solution 67 parts + 50 per cent. solution of sodium nitrite, 33 parts, 5 minutes. The light band in this preparation is so narrowed as to be practically indistinguishable except when the fibres break at this point × 1000.

Fig. 26.—Wing Muscle, *Aeschna.* Cobalt solution 50 parts + 50 per cent. sodium nitrite, 50 parts, 2 hours. Central zone of the dim band free from potassium holding material. Light band also shews no reaction × 1000.

Fig. 27.—Muscle Fibre, *Calosoma Scrutator.* Cobalt solution 50 parts + 50 per cent. sodium nitrite, 50 parts, 2 hours × 1000. Central third of dim band free from potassium.

Fig 28.—Wing Muscle, *Tenebrio molitor.* Cobalt solution, 50 parts + 50 per cent. sodium nitrite, 50 parts, 5 minutes. Shewing the localization of potassium at the junctions of the light and dim bands × 1000.

Fig. 29.—Wing Muscle, *Tenebrio molitor.* Cobalt solution, 15 hours. Reaction obtaining only on the borders of the light band × 1000.

Fig. 30.—Wing Muscle, *Tenebrio.* Cobalt solution, 15 hours. The distribution of the potassium wholly in the light band × 1000.

Fig. 31.—Wing Muscle, *Tenebrio.* Cobalt solution 50 parts + 50 per cent. solution of sodium nitrite, 50 parts, 5 minutes. Shewing a fibre as a wave of contraction is passing over it × 400.

Figs. 32-38.—Preparations treated with $\frac{N}{10}$ $AgNO_3$.

Fig. 32.—Muscle, *Lachnosterna fusca*. $\frac{N}{10}$ $AgNO_3$, 40 hours × 1000.

Fig. 33.—Thorax Muscle, Bee. $\frac{N}{10}$ $AgNO_3$, 5 days × 1000.

Fig. 34.—Thorax Muscle, Bee. $\frac{N}{10}$ $AgNO_3$, 5 days × 1000.

Fig. 35.—Wing Muscle, Wasp. $\frac{N}{10}$ $AgNO_3$, 18 hours × 1000.

Fig. 36.—Wing Muscle, *Calosoma*. $\frac{N}{10}$ $AgNO_3$, 60 hours × 1000. Phosphates, distributed in the borders of the dim band adjacent to the light bands × 1000.

Fig. 37.—Wing Muscle, *Desmoceros palliatus*. $\frac{N}{10}$ $AgNO_3$, 4 months × 1000.

Fig. 38.—Wing Muscle, *Benacus grisea*. $\frac{N}{10}$ $AgNO_3$, 10 days × 1000.

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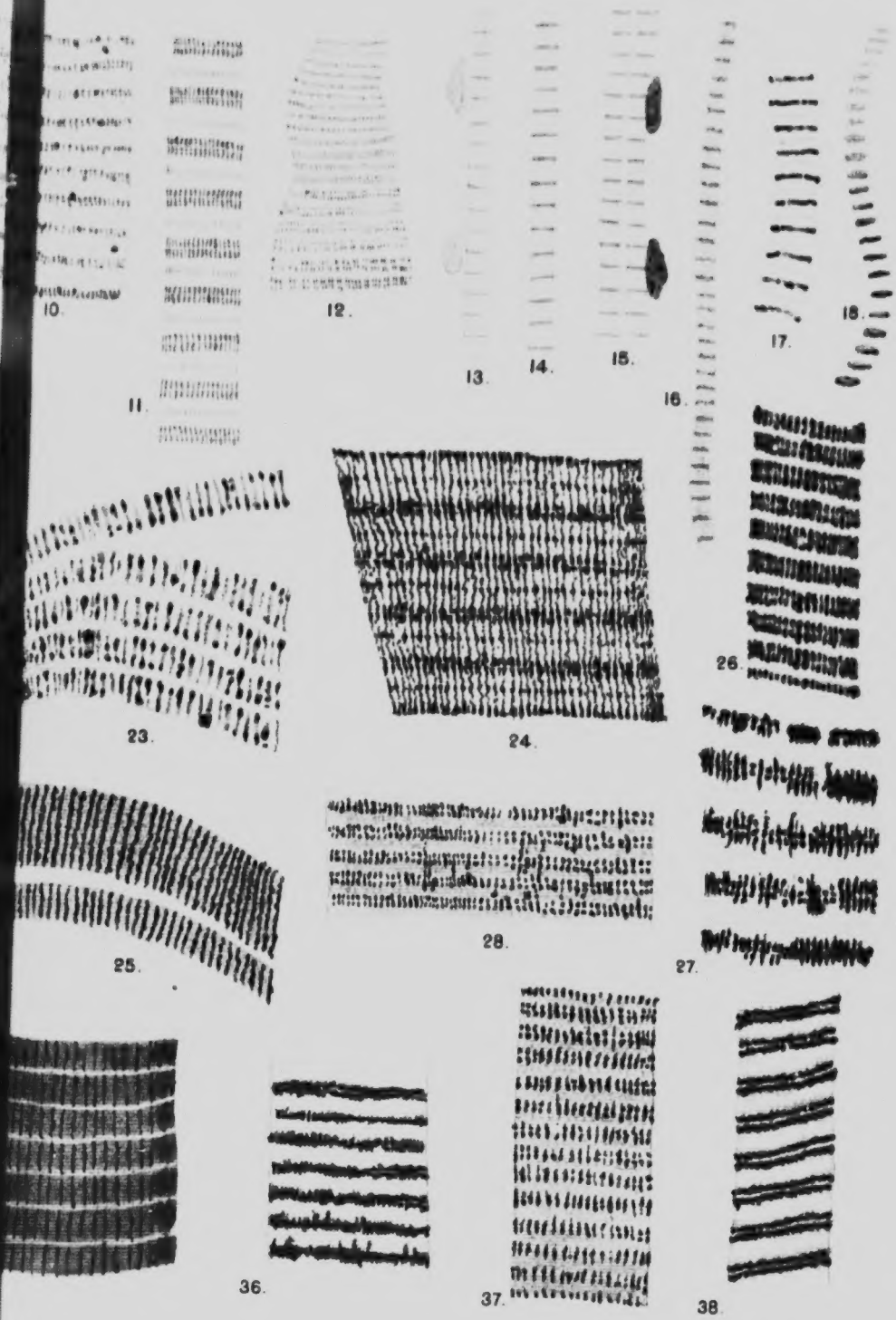

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